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13. ABSTRACT (Maximum 200 Words)

Androgens and the androgen receptor (AR) play a critical role in the development and progression of prostate cancers. The majority of prostate cancers initially respond to endocrine treatment (androgen dependent), but eventually become androgen independent that prove fatal. It appears that a functionally active AR may contribute to the progression of androgen-independent prostate cancers. Understanding the signaling pathways that regulate androgen-dependent and -independent activation of AR mediated transcription would provide valuable information for finding an ultimate cure for this disease. We have proposed to identify the signaling components that regulate AR activity using a novel retrovirus-mediated genetic system, and to understand the mechanism of how the identified factors control AR activity. To this end, we have established and optimized our retrovirus-mediated genetic screen approach. The retroviral vectors to be used have been improved which will allow high-efficiency mutagenesis and ease of manipulation and analysis. We have also made significant progress towards optimizing our screening strategies and establishing a suitable cell line for the genetic screen. With these tools in hand, we are in the process of performing the genome wide genetic screen to identify genes that are important for androgen receptor signaling and study how they may contribute to the progression of prostate cancers.

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Introduction

Androgens play a critical role in regulating prostate development and the evolution of prostate cancer (1). Androgens exert their biological function by activating gene transcription through the androgen receptor (AR), a member of the steroid nuclear receptor superfamily (2). Initially, endocrine treatment of prostate cancer patients either by inhibition of AR/androgen binding or deprivation of androgens can result in tumor regression. However, most prostate cancer patients relapse as the tumors become androgen independent (1, 3, 4, 5). An important step towards prostate cancer remedies is to understand the signaling pathways that regulate androgen-dependent and -independent activation of AR-mediated transcription. We propose to utilize a genetic system to screen for genes that regulate AR activity in cultured cells through high efficiency mutagenesis using Enhanced Retroviral Mutagens (ERM)(6). The overall objective of this proposal is to understand the molecular machinery that regulates AR transcription in normal and tumor prostate cells. We will first establish a genetic screening method to systematically search for genes that modulate AR activity *in vivo* using the PSA (prostate-specific antigen) enhancer and ARE as a reporter. How the identified genes regulate AR transcription activity and subcellular localization in the context of chromatin will then be investigated.

Body

For Task 1, we have proposed to isolate genes that are involved in regulating the activity of androgen receptor. To accomplish this, we proposed to generate a HeLa-AR cell line that would express luciferase and FKBP-caspase driven by ARE, and to use this cell line for genetic screens using engineered retrovirus mutagens. The rationale is to isolate clones of cells that can escape androgen-dependent caspase-mediated apoptosis as a result of retrovirus mediated mutagenesis. Subsequently, the gene loci that have been mutated to confer androgen independence in these clones can be identified.

To achieve this goal, we have have generated a stable HeLa cell line that expresses the androgen receptor (pCMC-AR). These cells were futher engineered to express GFP-NTR (Green fluorescent protein-nitroreductase) (7). NTR allows drug selection against GFP-NTR expression. In the presence of metronidazole (MN), NTR-expressing cells will die. We then went on to test the feasibility of using these cells for the genetic screen. The rationale was that we would sort GFP^+ and GFP^- cells respectively after MN treatment. To our disappointment, it was impossible to isolate GFP^- cells after adrogen treatment. Androgen stimulated GFP expression should be transient. However, we found that the HeLa-GFP cells remained GFP+ long after R1881 treatment.

We reasoned that our failure to isolate GFP^- cells made it impossible to use cell death as one of the criteria for isolating mutant clones. We therefore modified our screening strategy. As delineated in Figure 1, we will take advantage of the tetracyline-responsive promoter engineered into the ERM vectors (6). This promoter is turned off in the presence of tetracycline (tet-off).

Briefly, we will sort by FACS GFP^{hi}-AR HeLa cells. These cells are then used for infection with the ERM viruses. Successful ERM integration into the genome may result in lowered GFP expression if the ERM activates a repressor of the AR. We then will sort for GFP^{lo} expressing cells and expand these clones. These GFP^{lo} cells will then be maintained in the presence of tetracycline, which should turn off the tet-off promoter and lead to decreased levels of the

putative repressor. Such decrease will in turn alleviate the repression of GFP expression and results in an increase in GFP levels. Clones that exhibit such a shift in GFP expression (from GFP^{hi} to GFP^{lo}, and then to GFP^{hi}) will be expanded and analyzed to clone the gene targeted by ERM. We are currently in the process of finalizing the conditions for the genetic screen using this strategy.

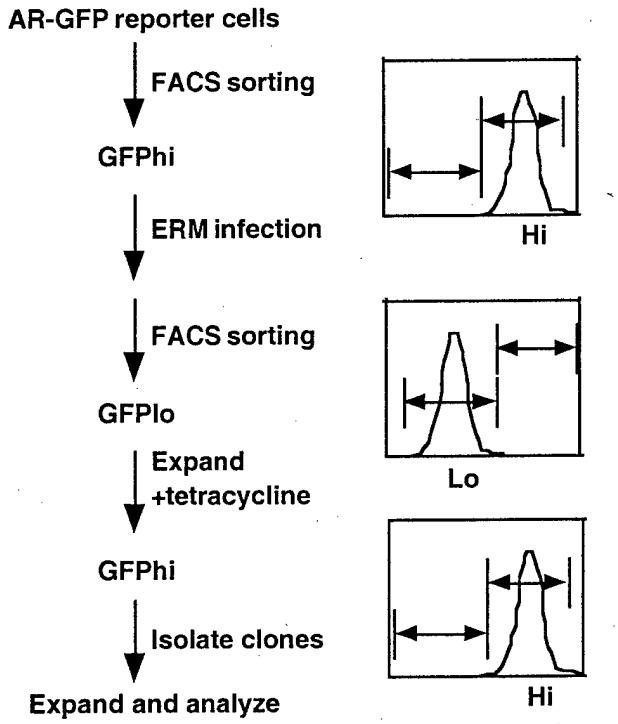


Figure 1. ERM-mediated genetic screening strategy.

In the mean time, we have also optimized our ERM vectors to be used in the genetic screen. We have modified the epitope tags and promoter regions to improve the mutagenesis efficiency and ease of manipulation. A collectioin of these constructs are illustrated in Figure 2. Because Task 2 is dependent on Task 1, we expect to make significant and new progress once Task 1 is completed.

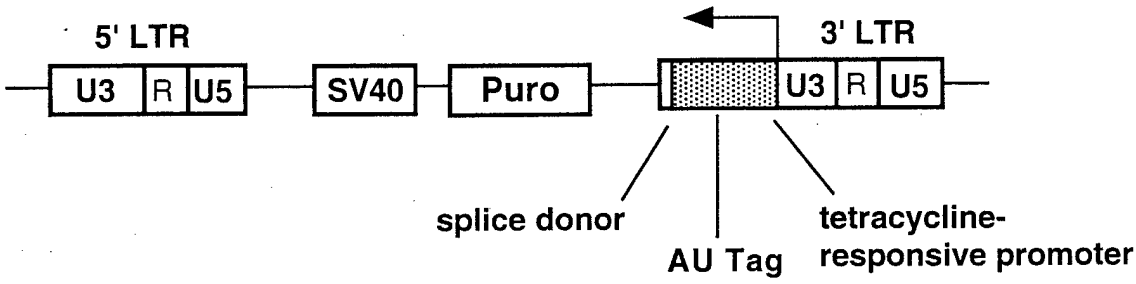
Key Research Accomplishments

- Improvement of the ERM genetic screen approach
- Improvement of the cells used for the ERM screen

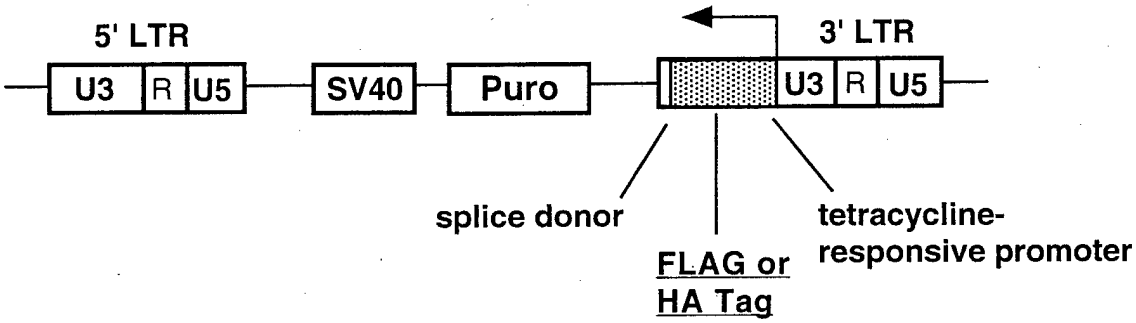
Reportable Outcomes

In our continued effort to improve genetic screen efficacy and efficiency, we have further improved the retrovirus based genetic screen vectors. These new ERM vectors should provide high efficiency mutagenesis. Furthermore, they should provide easier makers and epitopes for analysis and manipulation. At the same time, we have modified our screening strategies to allow for successful genetic screen.

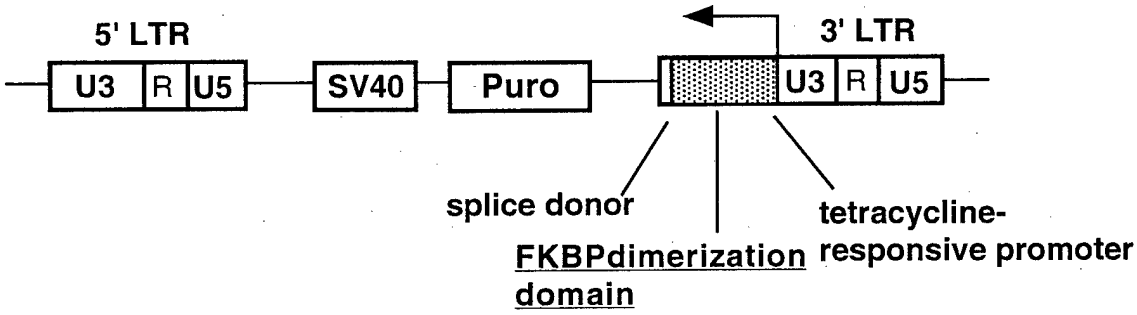
ERM



ERM-HA/FLAG



ERM-FKBP



ERM-puroSD

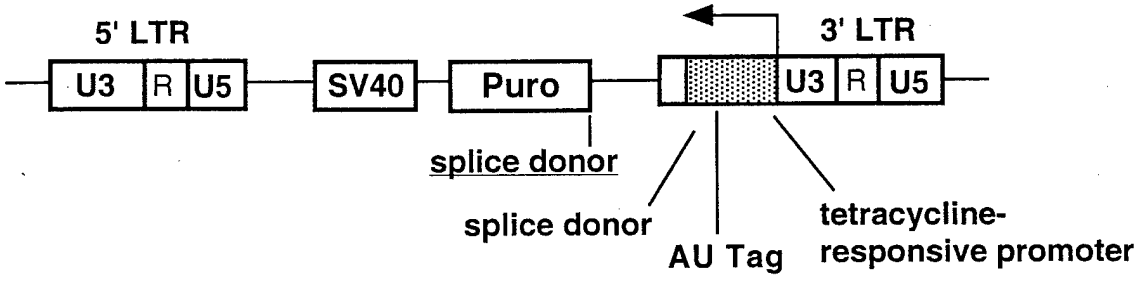


Figure 2. Schematic representation of various ERM vectors

Conclusions

In summary, we have further improved our genetic screen approach for high efficiency mutagenesis. Our progress was somewhat hampered due to difficulty in generating a suitable cell line to use for the genetic screen, we have overcome this difficulty by modifying our screening strategies. We are well on our way to starting the genetic screen and obtaining mutant clones. The genes that have been mutated in these clones will then be identified and studied to examine how they may affect the androgen receptor signaling pathway. Such information will prove invaluable in our quest for the cure of prostate cancers.

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